

Seropositivity rates of zoonotic pathogens in small ruminants and associated public health risks at informal urban markets in Zambia

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ABSTRACT

Informal livestock markets are an important source of animal-derived proteins for growing urban populations in countries such as Zambia. In parallel, they can also constitute pathways of zoonotic pathogen transmission to humans. This risk is aggravated by limited disease monitoring and poor control systems with regards to biosecurity and public health. The aim of this study was to investigate the risks for spread of zoonotic diseases in Zambia's two largest informal small ruminant markets, located in Lusaka and Kasumbalesa, through combining seroepidemiology with interviews and observations. In April, May and September 2018, serum samples ($n = 237$) were collected and analysed for antibodies for the zoonotic pathogens *Brucella* spp., *Coxiella (C.) burnetii* and Rift Valley fever virus (RVFV), using commercially available enzyme linked immunosorbent assays (ELISA). In addition, slaughterhouse activities were observed and semi-structured interviews and focus group discussions held with slaughterhouse workers and small ruminant traders, focusing on the handling of animals and meat, and the perceptions of zoonotic disease risks at slaughter and consumption. The study found seropositivity rates of 10.1% (95% confidence interval [CI] 6.60–14.7) for *Brucella* spp., 5.9% (95% CI 3.27–9.71) for *C. burnetii*, and 0.8% (95% CI 0.10–3.01) for RVFV. Interviews with value chain members and observations at the slaughterhouse revealed unsanitary procedures and multiple occupational hazards for slaughterhouse workers. This study showed that the Zambian informal small ruminant trade system poses risks to public health, and that these risks are exacerbated by a lack of information about food-borne diseases and how associated risks can be mitigated amongst value chain actors. The results of this study can be used to formulate preventive measures to improve informal meat markets and reduce the risks to public health.

1. Introduction

The demand for animal-derived proteins has increased in recent years, most notably in low- and middle-income countries such as Zambia. Informal urban livestock markets play an important role in meeting this demand (Hichaambwa, 2012), but can also facilitate the spread of zoonotic pathogens and pose severe risks to public health. There are numerous examples of outbreaks of zoonotic disease in humans linked to animal markets, e.g. Q-fever (Porten et al., 2006), avian influenza (Wan et al., 2011; Mounts et al., 1999) and, currently, COVID-19 (Huang et al., 2020). These markets may also facilitate the

spread of food-borne pathogens. These issues are exacerbated by a lack of organised routine disease monitoring systems, poor biosecurity and insufficient public health control mechanisms within the informal market sector.

Zambia has experienced one of Africa's fastest urbanisation rates (The World Bank, 2021), which in turn has led to increased demand for animal-derived proteins in urban areas. In response, informal and unplanned trading areas have mushroomed more quickly than the rate at which the Zambian government has been able to offer organised formal trading areas. The sheep and goat populations in Zambia are approximately 3.6 million and 170 000, respectively (Ministry of Fisheries and Livestock, 2019). Small ruminants play multiple important roles, for

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Abbreviations

RVF	Rift Valley fever
RVFFV	Rift Valley fever virus
ELISA	Enzyme-linked Immunosorbent Assay

example as a source of monetary income, food and to increase household resilience to internal and external shocks (Namonje-Kapembwa et al., 2016). There are several small ruminant markets in Zambia, but most sheep and goat trade is conducted at the two largest informal small livestock markets: Lusaka market, which is situated in a township in the capital, and Kasumbalesa market at a border crossing-point to the Democratic Republic of the Congo (DRC) (Namonje-Kapembwa et al., 2016; Lysholm et al., 2020).

There is an array of zoonotic sheep and goat pathogens that can be transmitted to humans during typical market-related activities, e.g. at slaughter, by close proximity to an infected animal or through consumption of meat or offal. However, research is limited on the occurrence of zoonotic pathogens in small ruminants in Zambia (Davies et al., 1992; Qiu et al., 2013; Muma et al., 2006; Hussein et al., 1985). Brucellosis is a zoonotic disease caused by several species within the bacterial genus *Brucella*. In small ruminants it is typically caused by *Brucella* (*B.*) *melitensis*, *B. abortus* or *B. suis*, as well as *B. ovis* in sheep (Díaz Aparicio, 2013), of which all except *B. ovis* have zoonotic potential (Seleem et al., 2010). Humans can become infected through contact with body fluids at for example slaughter, consumption of unpasteurised milk and undercooked meat, or through contact with placenta, foetal fluids or vaginal discharge from infected females (Seleem et al., 2010; Casalinuovo et al., 2016). Q-fever, coxiellosis, is a zoonotic disease caused by the bacterium *Coxiella* (*C.*) *burnetii* (Eldin et al., 2017). Humans can become infected by aerosol spread when in proximity to an infectious individual, e.g. at animal markets (Porten et al., 2006) or at slaughter (Eldin et al., 2017). Rift Valley fever virus (RVFV) is a zoonotic pathogen that typically appears in epizootic outbreaks at intervals of approximately 5–35 years (Dautu et al., 2012). It is spread by certain species of mosquitoes and through contact with body fluids, tissues and organs from infected animals. Humans are at risk of infection, for example when slaughtering animals, processing meat or organs, or consuming undercooked meat (Chevalier et al., 2010). RVFV has been described as endemic in Zambia and has been detected all over the country (Davies et al., 1992; Ghirotti et al., 1991; Samui et al., 1997; Hussein et al., 1985). However, there has been no reported clinical case of RVF in Zambia in more than three decades (Dautu et al., 2012).

The informal market sector provides food for a large part of the population but can pose severe risks for public health, for example through occupational exposure of livestock value chain members to zoonotic pathogens. Slaughterhouse workers, particularly those working in informal livestock value chains, are at increased risk for exposure to several zoonotic pathogens, including *Brucella* spp., *C. burnetii* and RVFV (Ikegami and Makino, 2011; Eldin et al., 2017; Seleem et al., 2010; Swai and Schoonman, 2009; Marrie and Fraser, 1985; Awah-Ndukum et al., 2018; Nabukenya et al., 2013). In addition, the sector can contribute to the spread of food-borne diseases. Although largely neglected, food-borne diseases cause a considerable burden to both the individual and society (Havelaar et al., 2015; Jaffee et al., 2019). The risk for contamination of meat and offal is high at slaughter and carcass dressing procedures and, therefore, ensuring that these processes are conducted in a safe and hygienic manner is of essence.

The overall aim of the current study was to investigate the risks of the spread of zoonotic diseases at Zambia's two largest informal small ruminant markets: Lusaka and Kasumbalesa markets. Specifically, one objective was to investigate the seropositivity rates and risk factors of zoonotic pathogens (*Brucella* spp., *C. burnetii* and RVFV) in small

ruminants at the markets. Another objective was to document slaughter routines, procedures and hygiene at a market slaughterhouse to understand the risk of the spread of zoonotic pathogens through occupational exposure at slaughter, as well as risk for contamination of meat and viscera resulting in compromised food safety. Lastly, the study sought to map perceptions of zoonotic foodborne disease risks and practices employed to mitigate these risks amongst small ruminant traders, to better understand the risk of disease through consumption.

2. Materials and methods

2.1. Description of the markets

The study was conducted in April-May and September 2018. In April-May, semi-structured interviews, focus group discussions and observations were performed at the Lusaka market, and in September, serum sample collection, semi-structured interviews and observations were conducted at the Lusaka and Kasumbalesa markets (Fig. 1).

The Lusaka market is situated in the Chibolya compound and consists of one main market area and two small and less active markets that are situated deeper into the compound. For practical reasons, only the big main market was included in this study. The Kasumbalesa market consists of three approximately equally sized trading areas, all of which were included in this study. In both markets, goats, pigs, sheep, chicken, and other fowl were sold. The small ruminants found at the market places are sourced from across the country, with the majority originating from Southern province (Namonje-Kapembwa et al., 2016). At both markets, goats were considerably more common than sheep. Co-transportation of goats, pigs, sheep and fowl from the same area is common, and after arrival at the markets, they are off-loaded into pens in close proximity to each other but separated by metal fences (Lysholm et al., 2020). Access to clean water to e.g. wash hands and clean tools was limited at both market places. In addition to animal pens, the Lusaka small livestock market also had a veterinary shop (i.e. a store where veterinary drugs can be purchased) and two slaughterhouses: one for small ruminants and one for pigs. At the Kasumbalesa market, there was no veterinary shop or designated slaughterhouse at the time of the study visits. Market activities were highly seasonal, with more trade occurring towards the end of the month, around celebrations and holidays, as well as prior to the due date of school fees. All market visits conducted as part of this study occurred at times of relatively low trade activity.

The number of small ruminant traders present at the Lusaka market varied greatly, ranging from approximately 10–30 per visit. In general, the same traders were present at the markets during most of the study visits and were occasionally joined by temporary traders. There was also considerable variation in the number of sheep and goats at the market, ranging from around 70–200 sheep and goats kept in around 10–14 pens. Due to the short amount of time spent at the Kasumbalesa market, we cannot estimate the number of traders and animals present there. As the focus for the data collection was to understand the organisation of the trade, the trade environment and the traders and slaughterers perspectives, we did not note down the exact numbers of traders and animals present.

2.2. Serology

The target population for the serology study was small ruminants present at the Lusaka and Kasumbalesa markets in September 2018, and the study was designed to provide a cross-sectional view of the seropositivity rate of *Brucella* spp., *C. burnetii* and RVFV in these animals. The Lusaka market was visited on three occasions over two weeks, and the Kasumbalesa market once. Blood was collected from the animals' jugular vein using sterile needles and vacutainer tubes without additives. To avoid the same animal being sampled twice, the traders were asked to identify any animals that had previously been sampled and the skin in the area of the jugular veins was checked carefully for needle marks.

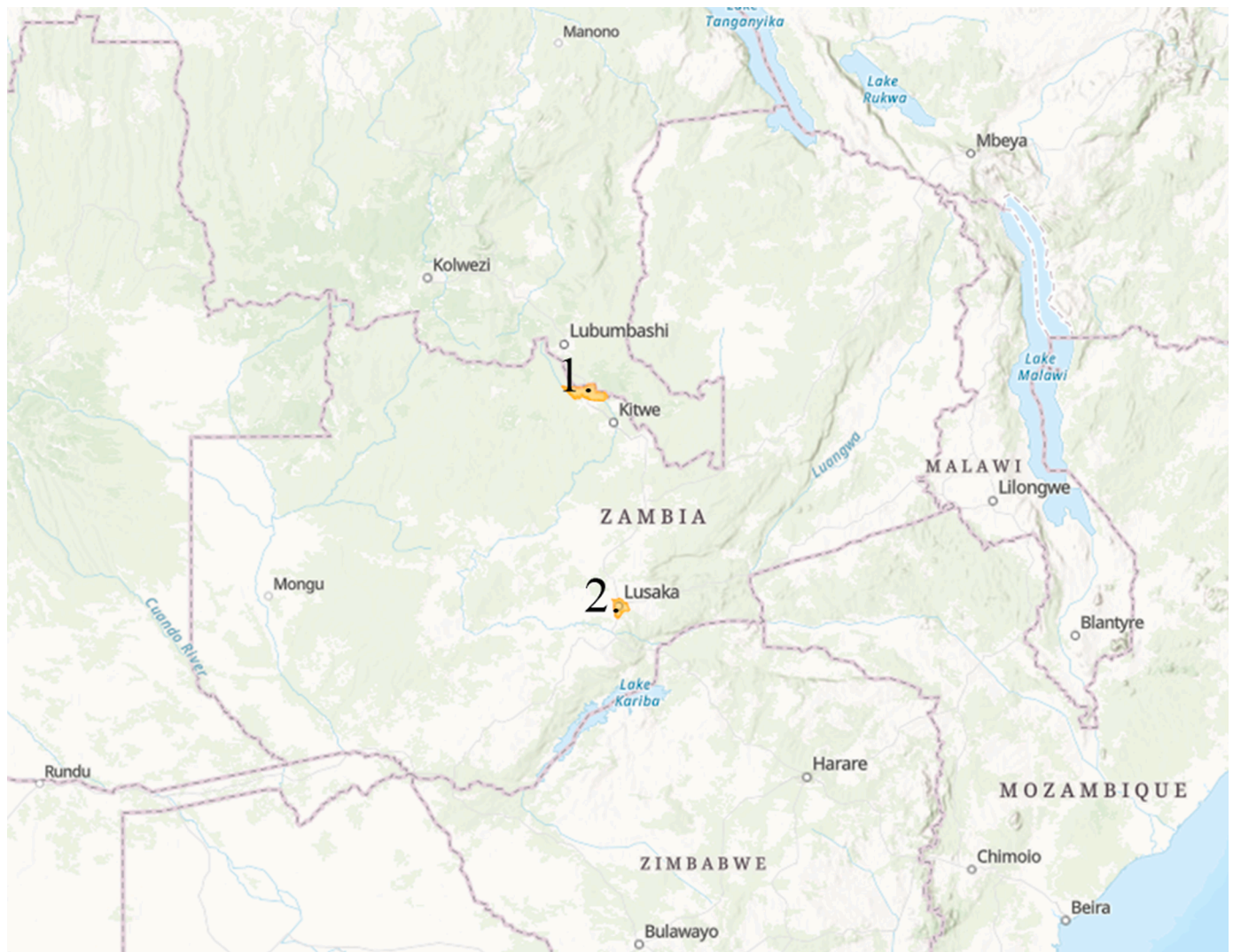


Fig. 1. Map depicting the positions and outlines of the districts where the two surveyed markets are located. 1=Chililalombwe district, containing the Kasumbalesa small livestock market. 2=Lusaka district, containing the Lusaka small livestock market. Source: Esri, USGS | Esri, © OpenStreetMap contributors, HERE, Garmin, FAO, NOAA, USGS.

After collection, the samples were placed in a vertical position in a cooler box to coagulate and allow serum to separate. Later the same evening, the serum was transferred to cryotubes and stored at -20°C until transportation to the laboratory, where samples were stored at -80°C until analysis. Species, breed, sex, origin and whether the animal was displaying any clinical signs of disease, was recorded for each sampled individual.

The study was based on a convenience sample scheme and samples were collected non-randomly. Prior to the study, a sample size of 385 small ruminants per market was calculated, applying a 5% precision, 95% confidence interval and estimated true prevalence of 50% to yield the largest necessary sample size. An infinite population was assumed as numbers on the size of the source population to the Lusaka and Kasumbalesa markets are difficult to find. However, collecting such a large number of samples randomly was not possible in the field, and hence the plan shifted to collecting as many samples as the traders would allow us to, and from estimating seroprevalence to the rate of seropositivity. Almost all the traders present at the Lusaka market and the majority of the traders present at the Kasumbalesa market on the dates when blood samples were taken, were approached and asked for their verbal consent to participate in the study. Approximately 5–10% declined participation, with the most commonly stated reason being lack

of time or energy, and many agreed to participate at a later time instead. The selection of animals was non-random because it was the trader who chose the animals from which samples would be collected. This was generally a prerequisite for the traders to grant their permission. Some traders would allow us to obtain serum samples from all the sheep and goats in their pens, however, the majority limited this number to five or less.

Samples were analysed for the presence of antibodies with the following commercial enzyme-linked immunosorbent assay (ELISA) kits: *Svanovir Brucella-Ab C-ELISA* (detecting antibodies for *B. melitensis*, *B. abortus* and *B. suis*, reported sensitivity 100%, specificity 100%; Boehringer-Ingelheim Svanova diagnostics, Uppsala, Sweden), ID Screen Q-Fever Indirect Multi-species (reported 100% sensitivity, 100% specificity; ID-vet, Grabels, France) and ID Screen Rift Valley Fever Competition Multi-Species (reported sensitivity 100%, specificity 100%; ID-Vet). In addition to the tests done by the manufacturers, the *Brucella* spp., and RVFV ELISAs have been evaluated independently. These studies estimated the sensitivity and specificity of the *Brucella* spp. ELISA to be 99.4% and 98.9%, respectively (Biancifiore et al., 2000), and for RVFV 91–100% and 100%, respectively (Kortekaas et al., 2013). All the kits were used, validated and interpreted according to the instructions provided by their manufacturers. For RVFV, results could be

positive, negative or doubtful. Doubtful samples were considered negative in the statistical analysis.

2.3. Statistical analysis

Estimated true rate of seropositivity was calculated based on the apparent seropositivity rate and the sensitivity and specificity of the diagnostic tests, using EpiTools “Estimated true prevalence and predictive values from survey testing” in accordance with Rogan & Gladen (1978). The sample results were analysed for possible predictor variables associated with seropositivity, using Stata IC 16.1 (StataCorp LLC, USA). As the traders only had limited information about each animal, the included predictor variables were market, species, sex and provincial origin. Univariable analysis was conducted using Chi2 tests or Fischer’s exact test where applicable. The odds ratio was calculated using logistic regression. Multivariable analyses were not performed since only one predictor variable each for *Brucella* spp. and *C. burnetii*, and no predictor variable for RVFV, had a p-value of 0.25 or less in the univariable analyses. In addition, difference in origin of the sampled small ruminants at each market was analysed using Fischer’s exact test.

2.4. Semi-structured interviews, focus group discussions and observations

Qualitative data were collected at the Lusaka market over a total of 21 days split between April, May and September 2018, and on four days at Kasumbalesa market in September 2018.

Semi-structured interviews (Robson, 2011) were conducted by the first author with workers at the Lusaka market slaughterhouse and with sheep and goat traders, as described in more detail in Lysholm et al. (2020). The interviews were based on topic guides, i.e., lists of topics to be covered during the interview, while offering the opportunity for unexpected information from the participants to be followed up. The interviews with slaughterhouse workers covered themes such as workers’ perceptions and practices related to slaughter routines, procedures and hygiene. In addition, small ruminant traders were interviewed regarding perceptions and practices related to consumption and associated risks to human health. All the interviews were conducted with an interpreter and the responses noted down by hand by the first author, before being double-checked and completed in discussion with the translator directly after each interview. In Lusaka, most interviews were conducted in the local language of Nyanja, while in Kasumbalesa, Bemba was most commonly spoken. A small number of interviews were performed in Tonga or French; as the interpreter did not speak either language, others present at the market interpreted instead. Since these interviews were performed with an interpreter who lacked training and experience, they were judged to be of lower quality, which was later taken into account in the analysis. The quotations in the text are not verbatim but based on written notes. The meaning and essence of the informants’ words, as supplied by the interpreter, have not been altered.

The interview participants were chosen with purposive sampling strategies (Conroy, 2005). At the Lusaka market, five interviews with slaughterhouse workers and 35 with traders were performed. At the Kasumbalesa market, 12 traders were interviewed. As the vast majority of market workers and visitors were men, less than fifteen percent of the respondents in this study were women. Most interviews with the slaughterhouse workers were conducted in groups, with input collected from all the workers present who assisted each other in answering the questions. In all, approximately fifteen workers were estimated to have participated in the five interviews.

In addition to the semi-structured interviews, two focus group discussions (Robson, 2011) were performed with slaughterhouse employees in Lusaka. One session was performed with supervisors and one with workers; both groups were convened by the slaughterhouse manager. The supervisor group consisted of five respondents throughout the focus group discussion, whereas the worker group started with eleven participants, but they were joined by several temporary participants for

parts of the discussion, taking the number of participants up to a maximum of 20. Members of the supervisor session had on average 15 years’ working experience at the slaughterhouse, and workers six years.

The focus group discussion were divided into two sections:

- 1) a group interview based on a topic guide that followed the same themes as the individual interviews, i.e. slaughter routines, procedures, hygiene and ante-mortem and post-mortem findings.
- 2) ranking exercises, where the participants were asked to list important aspects related to a number of themes, such as good slaughter hygiene, and subsequently rank the different listed aspects in order of importance.

The discussions were held in the local Nyanja language and were facilitated by a facilitator. The facilitator followed the topic guide while being open to following up any new information provided by the participants. The first author was present during both focus groups, and notes from the discussions were taken by the first author as well as by an assistant note-taker who is fluent in Nyanja.

To enrich understanding of slaughter routines, procedures and hygiene, and in particular to get more information on slaughterhouse practices (as what people say they do often represents a rather idealised portrayal of what is actually going on), the daily activities at the slaughterhouse were observed by the first author. The observations focused on documenting slaughter procedures and identifying steps at which meat and organ hygiene was compromised or where the slaughterhouse workers were put at risk of occupational exposure to pathogens. In total, five sessions of observations were conducted at the slaughterhouse, in general lasting for one to two hours. During the observations, detailed field notes were taken that were later rewritten. In addition, the activities were filmed with consent of the participants.

The data analysis was facilitated by the use of NVivo 12.2.0 software (QSR International, Warrington, UK) and was performed by the first author, guided by the third author. The notes from the semi-structured interviews, focus group discussions and observations were coded thematically in an iterative process guided by the research questions but inductively being inspired by the research material. Initially broad themes were created based on the focus for the research and the themes emerging from the data. Through repeated readings of the material, initial broad themes became more narrow and detailed (Miles and Huberman, 1994; Bowen, 2006). The coding process focused on identifying practices and perceptions amongst slaughterhouse workers that influenced their risk for occupational exposure and for organ and meat contamination at slaughter. Another focus was to map perceptions of risks for zoonotic foodborne disease and practices used to reduce these risks amongst small ruminant traders, to better understand the possibility for exposure to foodborne disease through consumption.

2.5. Ethical considerations

Before the work was initiated, market representatives were approached in order to explain the purpose of the study and obtain their permission to conduct the research. Prior to the interviews and animal sample collection, oral informed consent was obtained from each participant/animal owner. Care was taken to inform the participants of the voluntary nature of the project, that there were no repercussions if they chose not to take part, and that consent could be withdrawn at any time. Participant anonymity and confidentiality were ensured by only collecting personal details relevant for the study, and by never disclosing information related to individual informants to anyone outside the research team. Only individuals aged 18 and above were allowed to participate in the study.

The study received ethical approval from the International Livestock Research Institute’s (ILRI) Institutional Research Ethics Committee (IREC) (ILRI-IREC2018–04).

3. Results

3.1. Descriptive statistics

In total, 237 serum samples from sheep and goats were collected for the study. Of these, 143 samples (60.3%) were collected at the Lusaka market on three occasions over two weeks, while 94 samples (39.7%) were collected at the Kasumbalesa market on one occasion. Only 14 (5.9%) of the sampled animals were sheep, of which ten were sampled at the Kasumbalesa market and four at the Lusaka market. In total, 108 (45.6%) were male, 125 female (52.7%) and for 4 animals, the sex was not recorded. The majority of the sheep and goats were of local mixed breeds. Only six of the sampled animals displayed clinical signs at the time of the visits, including nasal discharge ($n = 4$), coughing ($n = 2$) and repeated sneezing ($n = 1$). The sneezing goat was seropositive for *Brucella* spp., although it is unlikely that brucellosis was the underlying cause of the animal's health issue. The other animals with clinical signs were seronegative for all three diseases.

The origin of the small ruminants sampled was significantly different between the markets ($p = 0.02$). While most animals originated from Southern province (78.0% at Kasumbalesa market vs. 91.0% at Lusaka market), Eastern and Central province were more common providers to Kasumbalesa market (11.4% and 10.2%, respectively) than to Lusaka market (3.7% and 4.4%, respectively). Only one animal originated from Lusaka province. The most frequently mentioned districts were Choma (36.0%), Monze (16.0%) and Kalomo (13.0%), all of which are in the Southern Province. For 13 (5.4%) of the sampled animals, the origin was unknown.

3.2. Serological results

The rates of seropositivity in this study was 10.1% (*Brucella* spp.), 5.9% (*C. burnetii*) and 0.8% (RVFV) (See Table 1). The manufacturers of the ELISAs utilized in this study reported sensitivity and specificity values of 100%, which means that the estimated true seropositivity rate is the same as the apparent rate of seropositivity. However, if the sensitivity and specificity estimates from two independent evaluations of the *Brucella* spp. and RVFV ELISAs are used, the estimated true seropositivity rates were 9.2% (95% CI, 5.9–13.8) for *Brucella* spp. and 0.9% (95% CI, 0.3 – 3.3) for RVFV.

One animal was seropositive for two different pathogens, i.e. *C. burnetii* and *Brucella* spp. No animal was seropositive for more than two pathogens.

3.3. Risk factor analysis

Analyses were conducted to investigate associations between seropositivity and market, species, sex and origin. These were conducted for each pathogen separately, as well as for seropositivity to at least one pathogen. The seropositivity rate of *Brucella* spp. was significantly higher at the Lusaka market than at the Kasumbalesa market, with an odds ratio of 3.66 (95% CI 1.21–11.1, $p = 0.01$). For *C. burnetii*, rate of seropositivity was significantly higher in animals that originated from Central Province and Eastern Province, with odds ratios of 15.6 (95% CI 0.88–26.1, $p < 0.01$) and 4.79 (95% CI 4.05–59.9, $p < 0.01$) respectively. No other potential risk factor was statistically significant.

3.4. Slaughter at the Lusaka market

The slaughterhouse for small ruminants was situated on the edge of the market square. It consisted of one room of approximately 80 m², with a partially demarcated area where customers waited, and some meat preparation took place. Slaughter occurred in the centre of the main room where animals were hung upside down prior to slitting the throat to bleed it. On the floor there was a sewage outlet for the disposal of blood, faecal matter, offal, water etc. This outlet was frequently clogged at the time of visits, and therefore the slaughterhouse workers often had to perform their work standing in dirty water. Along the walls there were benches where meat and organs were prepared. The benches were covered with pieces of cardboard that were replaced at the end of each day. A water container was positioned under a tap from where the slaughterhouse employees could collect water. The water in the tap was reported to be drinking water provided by the Lusaka city council. The slaughterhouse was heavily infested with flies and, due to the moist conditions, condensation would frequently form and drop down onto the meat, organs and people.

Approximately 15–20 people were working in the slaughterhouse at the time of our visits. Almost all the workers were men, with the exception of one woman who was not involved in slaughter or meat and organ preparation. In the interviews, the participants stated that they had no formal education but were trained at the workplace by shadowing an experienced worker. For most, the training lasted one or two days, while some needed up to two weeks before supervisors considered the person ready to work independently. The slaughterhouse workers worked under supervisors who were responsible for the slaughter and carcass dressing process. At the time of visits, the workers did not have access to personal protective equipment, such as plastic gloves or aprons. They wore their own work clothes which they reported to take

Table 1
Seropositivity at individual animal level for *Brucella* spp., *Coxiella burnetii* and RVFV, by market, species, sex and provincial origin .

		Samples analysed	<i>Brucella</i> spp.		<i>Coxiella burnetii</i>		RVFV	
			Positive	% positive (95% CI)	Positive	% positive (95% CI)	Positive	% positive (95% CI)
Market	Total	237	24	10.1 (6.60–14.7)	14	5.91 (3.27–9.71)	2	0.84 (0.10–3.01)
	Kasumbalesa	94	4	4.26 (1.17–10.5)	7	7.45 (3.05–14.7)	0	0 (0–3.85)
	Lusaka	143	20	14.0 (8.76–20.8)	7	4.90 (1.99–9.83)	2	1.40 (0.17–4.96)
Species	Goat	223	23	10.3 (6.65–15.1)	14	6.28 (3.47–10.3)	2	0.90 (0.11–3.20)
	Sheep	14	1	7.14 (0.18 –33.9)	0	0 (0–23.2) ^a	0	0 (0–23.2) ^a
Sex ^b	Female	125	15	12.0 (6.67–19.0)	5	4.00 (3.88–15.2)	2	1.60 (0.19 –5.66)
	Male	108	9	8.33 (3.88 –15.2)	9	8.33 (1.31–9.09)	0	0 (0–3.36) ^a
Province ^c	Southern	193	22	11.4 (7.28–16.7)	6	3.11 (1.15–6.64)	2	1.04 (0.13–3.69)
	Eastern	15	0	0 (0–21.8) ^a	2	13.3 (1.66–40.5)	0	0 (0–21.8) ^a
	Central	15	1	6.67 (0.17–31.9)	5	33.3 (11.8–61.6)	0	0 (0–21.8) ^a
	Lusaka	1	0	0 (0–97.5) ^a	0	0 (0–97.5) ^a	0	0 (0–97.5) ^a

^a One-sided 97.5% confidence interval.

^b Missing information for 4 animals.

^c Missing information for 13 animals.

home after each day to wash. Most slaughterhouse workers used gumboots, but some were seen working in sneakers or flip flops. The footwear was reportedly washed with soap at the end of each day. In addition, the workers said they disinfected the floor with chlorine at the end of the day before going home. No soap or disinfectant was however seen at the slaughterhouse.

The slaughterhouse workers reported that they in general slaughtered between two and four sheep or goats per person per day, but during busy periods this number could rise to ten animals a day. Peak periods were towards the end of the month and major holidays such as Christmas. Their regular customers were from butcher's shops, restaurants, hotels and bars, as well as market vendors and individuals buying for home consumption. When asked to describe the slaughter procedure, the workers reported that they commenced with negotiating a price with the customer, and when an agreement had been made, the animal was brought onto the slaughter slab. The animals were mostly hung upside down prior to having their throat slit, but it was occasionally observed how the workers cut the throat and restrained it on the ground while it bled. Following the bleeding, the slaughterer typically skinned the animal, and then opened up the abdomen and thorax to remove the internal organs, without prior tying of the oesophagus and colon. What happened after this depended on the customer and her or his wishes. There were different stations along the sides of the room where additional tasks could be performed, such as crushing bones with a sledgehammer or slicing the intestines into segments. In order to keep track of which body parts and organs came from which animal, the slaughterers would place them inside the pelt on the floor. According to the respondents, there was no opportunity for cold storage at the slaughterhouse.

The slaughterhouse workers used two different tools during the slaughter procedures, a bucket and a knife. The bucket contained tap water from the water container and was used to clean the removed intestines by dipping them in the water and letting it rinse through. As a result, water contaminated by faecal matter, blood and dirt was frequently observed. In addition, it was often seen how the workers used the contaminated water in the bucket to wash their arms and hands, as well as to scoop out water and throw it onto the carcass to wash away blood and fur. According to the workers, the washing procedure made the meat taste good, ensured a good reputation amongst customers, and prevented the meat from going bad as it is believed that consumers could get sick if they ate unwashed meat.

If we do not wash the carcass with water, the customers can get sick!

Slaughterhouse supervisor

The standard procedure was to discard the water and clean the bucket in between every sheep or goat. However, a few slaughterhouse workers said that they did this several times per animal, while others admitted that they only changed the water and cleaned the bucket every two or three goats. The water in the bucket would be thrown onto the floor and a worker would use the water and a wooden brush to remove dirt from the floor. The content of the bucket would then be poured down the drain.

The slaughterhouse workers reported that they used the same knife throughout the slaughter procedure and washed it in the bucket when it was considered dirty, which was generally after the throat was cut and the skin removed. Sometimes the workers were also observed washing the knife in water from the tap or to wipe it clean on the animal's pelt. The knives were sharpened using knife sharpeners that several workers shared. Although reportedly not permitted by the supervisors, the workers were often seen with the knife placed inside their boot shaft or inserted into one of the animal's hind legs. When the slaughterhouse workers were asked if they used soap to clean the knife or their arms and hands, they replied that they did not use soap as this would make the meat turn bad.

According to both the slaughterhouse workers and supervisors, no ante-mortem or post-mortem inspection by trained personnel was performed at the slaughterhouse. According to the workers, it was

uncommon for the animals to display clinical signs prior to slaughter, although ocular and nasal discharge would occasionally be observed. Also, it was rare for the slaughterers to discover macroscopic abnormalities on the carcass or organs after slaughter. The most common finding was ulcers on the intestines. The second most common finding was soft, watery lungs, sometimes filled with pus or sores on the lungs or in the thoracic cavity. The slaughterhouse workers also reported rare findings of enlarged livers with white and dark spots, sometimes containing endoparasites, reduced liver size, intestinal diarrhoea, rotten fetuses in wombs, and traumatic meat injuries. Upon noticing these post-mortem findings, the slaughterer would inform the customer and advise him or her to discard the damaged organ or meat defect. Most reported that they had never discarded a complete carcass, although during the focus group discussion with supervisors, it was mentioned that they would sometimes find goats that had suffered severe trauma prior to arriving at the slaughterhouse, e.g. during transport or at the market. In these cases, the meat was full of blood clots and had to be discarded and burnt as a result. According to two of the respondents, the buyer could demand a discount or a refund if parts of the meat and/or certain organs needed to be discarded.

The analysis of interviews with slaughterhouse workers generated two related dominant themes concerning what it means to be a good slaughterhouse worker, namely being quick and skilled at the slaughter procedure and maintaining good hygiene.

A good slaughterhouse worker is someone who is fast and clean. To be clean means to make sure that the bucket and knife is clean, and to wash the carcass often

Slaughterhouse worker, Lusaka

The importance of cleanliness was frequently emphasised by the slaughterhouse workers. If a worker failed to maintain proper hygiene, he would not attract customers, diseases could spread, and the slaughterhouse risked being closed down. The means to ensure good hygiene at slaughter that were mentioned included:

- personal hygiene (cutting nails, washing hair and body, washing hands, clean gumboots every evening and washing clothes daily)
- clean utensils and environment (using clean water, cleaning the bucket, knife and machete, disinfecting the floor with chlorine every evening)
- hygienic slaughter procedures (washing the meat and intestines and packing faecal material separately in a bag).

amongst these, most workers considered washing the meat and intestines as the most important step to ensure good slaughter hygiene.

3.5. Perceptions of zoonotic food-borne disease risks amongst market actors and risk mitigation measures

Most of the respondents, but not all, were aware of the risk of the spread of zoonotic disease associated with consuming animals displaying signs of disease prior to slaughter. Nevertheless, several respondents reported regularly consuming meat and offal from slaughtered sick animals. Consuming animals that had died a natural death was on the other hand rare, however, several respondents stated that this was common in their community. Many respondents believed that measures could be taken to eliminate the risks associated with consuming both sick animals and animals that had died a natural death. Examples include bleeding the animal thoroughly, boiling or drying the meat or discarding certain organs e.g. the intestines if the animal had diarrhoea.

There is no risk associated with eating the meat from a slaughtered sick animal, since the meat is always boiled first. I would however not eat an animal that died naturally, not even after boiling it, but I know several people who do.

Trader, Lusaka

Although not allowed at either market, a few traders reported selling the bodies of animals that had died a natural death. Some consumers

would say they were buying the carcasses to feed to their dogs, while others were open about buying them for human consumption. One respondent said he sold animals that had died naturally, mostly to barbeque meat stands outside bars selling food to people on their way home.

4. Discussion

The small ruminant market system in Zambia provides a source of income to a variety of value chain actors and supplies goat and mutton meat to the rapidly growing urban population. However, the system can contribute significantly to the dissemination of zoonotic and food-borne diseases. Given this scenario, this study combined sero-epidemiologic methods with interviews, focus group discussions and observations at the marketplaces to improve understanding of this risk.

Accordingly, the rate of seropositivity of the zoonotic pathogens *Brucella* spp., *C. burnetii* and Rift Valley fever virus (RVFV) was analysed, all three being pathogens about which there is little well-documented information in small ruminants in Zambia (Qiu et al., 2013; Muma et al., 2006; Hussein et al., 1985; Davies et al., 1992). Apparent rates of seropositivity were determined for *Brucella* spp. at 10.1%, for *C. burnetii* at 5.9%, and for RVFV at 0.8%. While being seropositive only indicates previous exposure and not active infection, the study findings suggest a continuous active circulation of *Brucella* spp., and *C. burnetii* in the markets' source populations. In a previous Zambian study, no small ruminants from various districts in Southern and Central Provinces were seropositive for *Brucella* spp. (Muma et al., 2006). However, seropositive cattle have been found in multiple studies in districts that are regular suppliers of small ruminants to the surveyed markets (Muma et al., 2013; Muma et al., 2006; Muma et al., 2007b; Muma et al., 2007a; Chimana et al., 2010; Mfunne et al., 2021). For *C. burnetii*, the results are in tandem with an earlier study in Zambia where the prevalence of the bacterial genetic material in goat blood was 7.5% in districts that regularly supply sheep and goats to the markets (Qiu et al., 2013). As both pathogens have a highly infectious nature and are capable of aerosolized spread (Seleem et al., 2010; Madariaga et al., 2003), this finding constitutes potential risks for multiple value chain members present at the market place. This risk is further illustrated by previous human outbreaks of *C. burnetii* in Germany linked to animal markets (Porten et al., 2006), and to slaughterhouse waste in France (Carrieri et al., 2002).

For RVFV, the rate of seropositivity was 0.84% (two positive female goats from Southern Province sampled at the Lusaka market), which is low and in agreement with the absence of disease reports in Zambia for the past three decades (Dautu et al., 2012). In Zambia, small ruminants often have a short lifespan, and it is hence unlikely that the animals sampled in this study had been alive during an epizootic RVF outbreak. However, evidence of interepidemic seroconversions in small ruminants has been found in for example Kenya (Mbotha et al., 2018), Tanzania (Wensman et al., 2015) and Zambia (Davies et al., 1992). Nevertheless, it cannot be ruled out that the seropositive animals in this study represented false positives.

The risk of contracting *Brucella* spp., *C. burnetii* and/or RVFV is higher amongst certain occupational risk groups, for example slaughterhouse workers (Ikegami and Makino, 2011; Eldin et al., 2017; Seleem et al., 2010; Swai and Schoonman, 2009; Marrie and Fraser, 1985; Awah-Ndukum et al., 2018; Nabukenya et al., 2013). At the Lusaka slaughterhouse, several practices were observed and reported that may contribute to an increased risk of the workers contracting disease. All the workers lacked access to basic abattoir personnel protective equipment such as plastic gloves and aprons, which in previous research has been associated with increased rates of seropositivity to *Brucella* spp. (Nabukenya et al., 2013; Awah-Ndukum et al., 2018; Esmaeili et al., 2016) and *Leptospira* spp. (Cook et al., 2017a). Personal protective equipment is particularly important for workers who are exposed to blood, ingesta and various bodily fluids, e.g. when bleeding the animal

(Swai and Schoonman, 2009; Abu-Elyazeed et al., 1996) and during the carcass dressing procedures (Cook et al., 2017a). In addition, slaughterhouse workers lacked easy access to hand-washing facilities and would instead clean their arms and hands in contaminated recycled water, which can lead to infection as well as pathogen spread to meat, organs or other slaughterhouse operatives. The absence of ante-mortem inspection services exposes the slaughterhouse workers to a wide array of sick animals (Cook et al., 2017a). This risk is exacerbated by the fact that previous research at the markets has demonstrated that the sale of animals displaying clinical signs is common, and that the traders are more likely to sell a sick animal for consumption rather than to a farmer for breeding purposes (Lysholm et al., 2020). Increased sale of animals in the event of a disease outbreak, as a way of salvaging some economic value before the animals are potentially lost to mortality due to disease, has been described in several studies in other countries (Lichoti et al., 2017; Mubamba et al., 2018; Chenais et al., 2017).

In addition, there is a considerable risk of the spread of food-borne and zoonotic disease within the informal market system in Zambia. The majority of the animals sold at the Lusaka and Kasumbalesa markets are sold for human consumption (Lysholm et al., 2020). One in ten of the sampled sheep and goats in this study were seropositive for *Brucella* spp., which can be transmitted to humans through consumption of undercooked meat for example (Casaliniuvo et al., 2016). At the slaughterhouse, meat is often sold to restaurants, hotels and market vendors, hence the potentially contaminated meat can reach and be consumed by large and diverse groups of people. The study also found several slaughter practices and infrastructural limitations that could contribute to the microbial contamination of food, in turn putting the consumer at risk of food-borne disease. No ante-mortem or post-mortem inspection was performed, and customers could demand a refund if discernible, macroscopic changes were discovered during slaughter, possibly making the slaughterhouse workers less inclined to reveal such findings. As the slaughterhouse consisted of only one room, the facility did not allow for separation of "dirty" (killing, bleeding) from "clean" (eviscerating and organ and meat preparation) operations. Dirty and clean procedures were performed by the same slaughterhouse worker without hygienic measures being taken when switching between them, and in close proximity to workers and carcasses at other stages of the slaughter and dressing process. The slaughterhouse workers also lacked formal training, which has been associated with increased bacterial contamination of carcasses in a previous study (Wamalwa et al., 2012). Hygiene was generally poor, equipment was not regularly washed or was washed in dirty water, and personal hygiene was deficient. In a previous study in Zambia, poor hand-washing routines have been linked to increased levels of *E. coli* and *Salmonella* spp. on poultry carcasses (Mpundu et al., 2019).

Despite the poor hygiene observed, the respondent slaughterhouse employees frequently emphasised the importance of hygiene at slaughter. One of the riskiest procedures that could compromise slaughter hygiene was the washing of intestines. This was often done with contaminated water, or resulted in contamination of the water that was later used to wash the carcass, the knife or the arms and hands of the slaughterhouse worker. Cleaning water has been shown to be a source of contamination in other studies in Zambia (Mpundu et al., 2019) and in Nigeria (Bello et al., 2011), where counts of different coliform bacteria increased from before to after washing, and bacteria were found in the water used for washing, indicating a risk of cross-contamination. In the data analysis, washing the meat and intestines was perceived as the most important steps to ensure good slaughter hygiene by the slaughterers. This perception can indicate a lack of understanding of disease transmission pathways and suggests a perception that water cleans away dirt regardless of the quality of the water. It should however be noted that the workers lacked access to appropriate equipment to hygienically remove faecal content from intestines, along with many other important structural necessities to ensure good slaughter hygiene. Limited understanding is hence not the only potential explanation for the observed

behaviours.

The risks of the spread of zoonotic disease are exacerbated by a lack of information and poor practices related to food-borne diseases and risk mitigation measures. In this study, the respondents seemed aware of the risks associated with consuming animals with signs of disease. Many believed however that this risk could be eliminated, for example through boiling, drying or sufficient bleeding of the animal at slaughter, a belief that also has been found in other studies in Zambia (Sitali et al., 2017). While these measures are important for ensuring food safety, they are not a guarantee that the food is safe to consume. Perceiving it as such may increase risk behaviours, such as the purchase of clinically ill animals for consumption.

Considerable research on slaughterhouses and their various risks to public health have been conducted in Sub-Saharan Africa, including in Zambia. However, previous research has tended to focus on the formal rather than informal sector. Similar conditions to those observed in this study also seem to exist in many formalised systems, such as a sub-optimal slaughter infrastructure, lack of ante-mortem and post-mortem inspections and unhygienic slaughter procedures (Cook et al., 2017a; Cook et al., 2017b; Swai and Schoonman, 2009; Komba et al., 2012; Nonga et al., 2010). However, the absence of disease monitoring and control for public health and biosecurity probably contributes to the informal sector carrying greater public health risks than the formal one. In view of the importance of the informal value chain for supplying animal-derived food, especially to poorer citizens, it is essential that this sector is also included in research and intervention programmes.

There are limitations to this study. The serum samples were collected non-randomly and the number of samples was small, 143 and 94 respectively. This needs to be taken into consideration when interpreting the serology results. Also, the small number of visits for data collection made the study vulnerable to temporary variations in source regions and disease outbreaks, for example. Furthermore the time between sample occasions was relatively short. As the exchange rate of animals at the markets is high, with the vast majority being sold the same or the next day, it was unlikely that the same animal was sampled twice in the study. To minimise this risk, the trader was asked to identify previously sampled individuals and the neck area was carefully examined to check for needle marks prior to sample collection. In addition, research data were collected at times of relatively low trade activity. As increased market activities increase the number of animals and people present, as well as the risk of stress-induced disease outbreaks, the risk of zoonotic disease outbreaks and spread is likely to be higher. Hence, visiting the markets during peak trade periods could have added valuable information for the study objectives. Lastly, when interpreting the results, the limitations in the study scope should be considered and while some of the study findings are relevant also for other, similar, informal small ruminant market systems and slaughterhouses, they are mainly applicable to the ones that were surveyed as part of this study.

5. Conclusion

This study confirmed the presence of antibodies to *Brucella* spp., *C. burnetii* and RVFV in small ruminants at the Lusaka and Kasumbalesa markets in Zambia. Furthermore, the results indicate continuous circulation of *Brucella* spp., and *C. burnetii* in the source population. Coupled with suboptimal procedures and hygiene at the Lusaka slaughterhouse and lack of information about disease risks and mitigation measures amongst value chain actors, clear risks exist for exposure to zoonotic pathogens as well as the spread of food-borne disease. Hence, the Lusaka and Kasumbalesa markets and the Lusaka slaughterhouse can have a serious negative impact on public health in Zambia. The results of this study could be used to formulate intervention strategies with the aim of reducing the risks for trade and market-related zoonotic disease spread, and as a basis for further research.

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CRediT authorship contribution statement

Sara Lysholm: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft. **Klara Fischer:** Formal analysis, Methodology, Supervision, Writing – review & editing. **Johanna F Lindahl:** Conceptualization, Formal analysis, Methodology, Supervision, Writing – review & editing. **Musso Munyeme:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing. **Jonas Johansson Wensman:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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